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ORIGINAL RESEARCH

# Using stable isotope analysis to determine the contribution of naturally occurring pond biota and supplementary feed to the diet of farmed Australian freshwater crayfish, redclaw (*Cherax quadricarinatus*)

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**Abstract** Nutritional requirements of redclaw (*Cherax quadricarinatus*) farmed in Australia are poorly understood and little is known on what is actively being consumed in semi-intensive pond culture. In this study the isotopic signatures of δ13C and δ15N of potential food sources were analysed with a multi-source mixing model to determine the extent of their contribution to the diet of farmed redclaw. Four commercial redclaw farms in North Queensland, Australia (Mareeba, Biboorha, Wondecla and Topaz) were sampled for naturally occurring pond organisms and commonly used supplemental feed such as raw corn, soybean, lupin, commercial redclaw, and chicken feed. Both naturally occurring pond biota and supplemental feed contribute to the tissue composition of redclaw to some degree. However, the contribution varies with the type of feed and availability of natural sources, for example plant material at Topaz Farm was a greater contributor with 43.9 ± 19.5 % compared to supplementary sources raw corn 8.20 ± 3.10 and lupin 1.60 ± 1.70 %. Moreover, some supplemental feeds provided a direct nutrient source for primary pond productivity; contribution of the redclaw pellet to zoo- and phytoplankton at Wondecla Farm was 83.1 ± 6.50 and 50.0 ± 9.50 %, respectively, with similarly high values for chicken feed at Biboorha Farm of 72.6 ± 4.70 and 83.4 ± 6.90 %. The cost effectiveness of such feeds needs to be questioned if these are not being consumed and utilised directly. Providing a species-specific formulated feed with improved water stability would enhance production reliability and facilitate growth within the industry.

**Keywords** Crayfish · Nutrition · Pond biota · Supplemental feed · Stable isotope analysis · Semi-intensive aquaculture

## Introduction

Redclaw (*Cherax quadricarinatus*) are a tropical species of freshwater crayfish native to Northern Queensland, Australia. Physically robust, tolerant of low dissolved oxygen concentrations, varied water quality conditions, relatively high stocking densities and with no planktonic larval stage production techniques are relatively straightforward (Jones 2002; Saoud et al. 2012; Thompson et al. 2003). Redclaw can grow to 200 g in 6–9 months under optimal conditions (Jones et al. 2002; Thompson et al. 2004) which is superior to many

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overseas commercially important species such as the Red Swamp Crayfish (*Procambarus clarkii)* which reaches up to 50 g in 7–10 months (Ackefors 2000; McClain et al. 2007). Further, approximately 30 % of the total body weight of redclaw is edible tail meat which is one of the highest yields compared to other farmed crustaceans (Thompson et al. 2005). These characteristics have seen redclaw exported to countries such as Argentina, Mexico, Spain, The United States, South-East Asia, and Central/South America (Food and Agriculture Organisation 2012; Rodríguez-Canto et al. 2002; Saoud et al. 2013). However, the industry is relatively small with total global production totalling 61.5 tonne worth USD$1.3million in 2011 (FAO 2012). In Australia, commercial aquaculture of redclaw is limited to a small number of farms in Queensland where production has continued to decline over the past decade. Only 41 tonnes worth AUD$862,000 was produced in 2010–2011 down from a peak of 100 tonnes in 2004–2007 (Heidenreich 2013). In comparison the global production (primarily China and the United States) of *P. clarkii* was 539,764 tonnes worth over USD$2.5-billion (FAO 2012). There is clearly a market for freshwater crayfish that Australian farmers have the potential to capitalise on if production can meet demand. However, limited information on fundamental nutritional requirements and the lack of an industry feeding standard has led to the stagnation of the redclaw industry and limits the ability of Australian farmers to increase production and compete on a global scale.

Current redclaw farming practices have remained fairly similar to when the industry began in the late 1980s. Farmed in earthen ponds, growth of redclaw is largely attributed to the consumption of naturally occurring pond biota. Farmers commonly promote growth of primary productivity with the use of fertilisers and detrital forage such as hay (Jones et al. 1996). These ponds are essentially small ecosystems that display many of the processes that occur in natural environments. Naturally occurring pond organisms such as zooplankton including cladocerans, copepods, chironomid larvae, rotifers, and eggs of other organisms, associated periphyton and other natural feedstuff are often thought to explain differences in growth rates and survival (Hernández-Vergara et al. 2003; Viau et al. 2012), providing adequate nutrition and inducing strong feeding responses (Duffy et al. 2011).

While the importance of primary productivity is clearly understood, supplemental feeding is a common component of intensive and semi-intensive farming practices. Currently there is no feed available that is formulated based on the nutritional requirements for redclaw farmed in Australia. Rather, a generic commercial feed modified from other aquatic species, particularly marine shrimp, is used (Saoud et al. 2012). Commercial chicken pellets, unprocessed legumes and/or food scraps are also often used as supplemental feed; however, this varies from farm-to-farm with the assumption that if a good crop is produced then the feeding regime must be adequate (Jones 1989; Saoud et al. 2012). Efforts to determine the nutritional requirements of crayfish species have increased in recent years with many studies looking at the suitability and digestibility of plant-based and other alternative protein sources to formulate cheaper and more nutritionally complete feed for culture (Campaña-Torres et al. 2005; Duffy et al. 2011; Pavasovic et al. 2007; Thompson et al. 2003, 2004, 2005). However, there has been little research conducted on what is actively being consumed by redclaw in commercial ponds under semi-intensive farming conditions. Understanding the contribution of naturally occurring organisms to the diet of crayfish and the role of supplemental feed is critical to advancing feed management strategies that will improve production efficiencies in pond aquaculture of redclaw.

Stable isotope analysis (SIA) is a method frequently used by ecologists to identify what food sources are assimilated by the organism and provides a comprehensive understanding of an organism's diet. Ingested material has a measurable ratio of heavy to light isotopes defining its isotopic signature (Fry 2007). The elements most often used in food web studies are carbon (13C and 12C) and nitrogen (15N and 14N) with both having two stable isotopes (Stenroth et al. 2006). Metabolic processing of ingested organic matter causes changes in the ratio of heavy to light isotopes (isotopic fractionation) of 13C:12C and 15N:14N which leads to distinct isotopic compositions (Jacob et al. 2005). The ratio of 13C:12C changes very little with increasing trophic level making carbon stable isotope analysis useful in indicating which primary producers are the important nutritional sources for consumers (Whitledge and Rabeni 1997). Similarly the 15N:14N ratio is a reflection of the consumers' food source with the enrichment of 15N consistent with increasing trophic level (Whitledge and Rabeni 1997). Thus, sources that contribute to growth can be identified by measuring the carbon and nitrogen stable isotope ratios of both the consumer and any of its potential food sources. The aim of this study was to use SIA to determine the extent to which naturally occurring pond organisms and supplemental feed contribute to the diet of *C. quadricarinatus* in commercial pond culture.

## Materials and methods

### Study sites

Field work was conducted across four farms located in the Atherton Tablelands region (17°16′S, 145°53′E) of North Queensland over 3 days. All four farms had different farming and feed management practices including the use of a variety of hides (e.g. old car tyres or milk crates, etc.), time and frequency of feeding, pond fertilisation methods, water source, and supplemental feed provided.

Mareeba Farm (17°01′37″S, 145°23′46″E) was situated in the semi-rural area of Mareeba. Ponds were all of the same size (approx. 45 m × 18 m × 0.5 m) and distribution across the farm area. Hides were old milk crates and hessian-type bags strung from ropes that ran across the pond. Unhulled raw soybeans were the only supplemental feed provided. Hay was used as a forage material at the beginning of the season.

Biboorha Farm (16°48′51″S, 145°31′33″E) is situated alongside the Barron River and in the middle of rugged bushland. Ponds were staggered along the hillslope making use of gravity fed water. Size and depth of the ponds varied slightly (approx. 53 m × 20 m × 1.5 m) with old tyres used as hides. Diamonnium phosphate (DAP) and hay were used as fertilisers. Supplemental feed was a combination of unhulled soybean and a commercial chicken feed.

Wondelca (17°18′34″S, 145°27′37″E) utilised natural spring and rain water to supply the farm. Pond size varied with the larger measuring approx. 53 m × 40 m and the average being around 40 m × 10 m. Depth was similar at 1.2 m. Supplemental feed was a combination of corn and a commercial redclaw pellet. Hay was also used as forage material at the start of the season.

Topaz Farm (17°24′18″S, 145°43′46″E) is located next to Mount Bartle Frere. Lush vegetation surrounded the ponds more so than was present at the other farms. Ponds were gravity fed with an approximate size of between 30 and 60 m (L) × 20 m (W) × 1.2 m (D). Supplemental feed was a combination of corn, unhulled lupin and the commercial redclaw pellet. A mixture of dolomite and lime was used as a fertiliser and was often combined with the supplemental feed during feeding.

### Redclaw sample collection

Traps were set by the farmer the evening prior to sampling. On arrival at each site traps were raised and the contents collected. Berried females were released back into the pond. The remaining crayfish from each pond were placed in a plastic bag and euthanized in an ice-water slurry. (Mareeba Farm $n = 22$, Biboorha Farm $n = 26$, Wondecla Farm $n = 24$ and Topaz Farm $n = 23$).

### Collection of feed samples

Two ponds at each farm ($n = 2$) were sampled for zooplankton, phytoplankton and any macro-invertebrates by dragging a 50 μm plankton net through the water column. The filtered material was then rinsed through a 500 μm mesh to separate any larger particulates, such as leaves and macro-invertebrates, before being stored in polyethylene containers (2 L per pond); sediment samples were collected by scooping approximately 2 cm of the topmost substrate layer into polyethylene containers (70 g per pond); any abundant plant matter seen growing in or around the pond margins was collected (100 g); hides were sampled for periphyton by scraping surfaces (30 g). Supplemental feed, fertiliser and forage material used by the farmers were also sampled (50 g of each feed source per farm).

All material requiring cold storage were kept in a 250 L esky filled with ice-water while in the field and then frozen to −20 °C on return to the lab while awaiting further preparation.

### Sample preparation

Redclaw were removed from the freezer and slightly defrosted at room temperature to remove excess ice. Crayfish were then individually weighed to the nearest 0.01 g with an average of: Mareeba Farm = 54.29 ± 14.36 g; Biboorha Farm = 33.09 ± 8.93 g; Wondecla Farm = 43.99 ± 23.94 g; and Topaz Farm = 42.83 ± 12.11 g.

Stable isotope analysis was determined for whole-body samples as well as tail-muscle samples. The first segment of muscle (closest to the abdomen, exoskeleton removed) was sampled and weighed to the nearest 0.01 g, with the remaining tail muscle material included in the whole-body sample. Whole-body and tail-muscle sub-sample were homogenised with a small sample (1 g) taken to analyse moisture content. Material for moisture content analysis was oven-dried (105 °C) to a constant weight.

Pond water was defrosted and filtered through a sequence of mesh sizes (>500 μm, 500–300 μm, 300–60 μm, and 60–20 μm) to separate into zoo- and phytoplankton. Particles 60–300 μm were considered to be zooplankton with filtration to 20 μm being phytoplankton (Duffy et al. 2011; Meakin et al. 2009) Where there was enough material from 300 to 500 μm this was treated as another individual sample. As disintegration or lysis comprises cell integrity samples were inspected under a light microscope prior to freeze-drying. No damaged cells were observed and samples were considered to be suitable for further analysis. Sediment samples were later thawed and sifted to remove rocks and larger particulates.

## Preparation for stable isotope analysis

Samples with high carbonate content such as the whole-body sample underwent additional treatment in preparation for carbon SIA (Feuchtmayr and Grey 2003). As the process to reduce the carbonate content can influence $\delta^{15}N$ values freeze dried whole-body, tail-muscle, and zoo- and phytoplankton samples were allocated into two groups. Those for nitrogen analysis were left untreated whilst those for carbon analysis were acidified by adding 2 M HCl drop-by-drop to 1 g material (with cessation of bubbling used as the indicator to determine the amount of acid to add (Bunn et al. 1995)). Acid treated samples were not washed with distilled water, instead HCl was removed by decanting after centrifuging the samples at 3000 rpm, a process which was repeated until removal of HCl was sufficient. This prevented a loss of $\delta^{13}C$ which can occur if washing in distilled water (after Carabel et al. 2006).

All samples were placed into −80 °C freezer 48 h prior to being freeze-dried for 24 h (Virtis benchtop 2 K, VWR, Australia). Dried samples were milled to a fine powder consistency. $\delta^{13}C$ and $\delta^{15}N$ values, carbon (%) and nitrogen (%) weight, and C:N ratios were determined using a Costech Elemental Analyzer fitted with a zero-blank auto-sampler coupled via a ConFloIV to a ThermoFinnigan DeltaV$^{PLUS}$ using Continuous-Flow Isotope Ratio Mass Spectrometry (EA-IRMS) at James Cook University's Advanced Analytical Centre (AAC), Cairns. Stable isotope results are reported as per mil (‰) deviations from the VPDB and AIR reference standard scale for $\delta^{13}C$ and $\delta^{15}N$ values, respectively. Precisions (S.D.) on internal standards were better than ±0.1 and 0.2 ‰ for carbon and nitrogen, respectively.

## Lipid extraction

Lipids are known to be depleted in $\delta^{13}C$, preferentially deposited as $\delta^{12}C$ (Bodin et al. 2007) with the removal of lipids routinely conducted, making samples isotopically heavier to better reflect the $\delta^{13}C$ of the diet in natural systems (Bunn et al. 1995; Stenroth et al. 2006). However, the resulting isotopic shift may be of little relevance if the effect on the estimated variable is negligible (Anthony et al. 2007). Further, diet estimates in a complex natural system (several sources) of a generalist consumer will likely be less impacted by isotopic variation compared to a specialist consumer in a simple (few sources) system (Tarroux et al. 2010). This will likely also be the case in an "artificial" food web of a semi-intensive aquaculture production system. To test this duplicate lipid and non-lipid extracted redclaw and source samples obtained from Mareeba Farm were compared. To extract lipid content samples were immersed initially in 100 % methanol and sonicated for 15 min, then in successive 2:1 Dichloromethane/Methanol solutions (each sonicated for 15 min), centrifuged and supernatant removed until clear. SIA was conducted as described above. No significant differences were noted for $\delta^{13}C$ between lipid and non-lipid extracted samples ($P > 0.05$). All subsequent SIA results are presented on non-lipid extracted samples.

### Stable isotope data analysis

#### *Multi-source mixing model*

A multi-source mixing model estimating the relative contribution of dietary nitrogen and carbon to source material was used to calculate the range of all possible source contributions in systems with two or more sources. (Isosource; Phillips and Gregg 2001, 2003). The multi-source mixing model iteratively creates each possible combination of source proportions (that sum to 100 %) in small increments (in this case 1 %). The predicted isotopic signatures for the mixture are compared with the observed signatures and if they fit within a small tolerance (i.e. 'mass balance tolerance'; Beatty 2006) it is therefore considered feasible and recorded. The resulting dataset represents all feasible solutions and distribution of proportions (Phillips and Gregg 2003). A mass balance tolerance of 0.1 % was initially used, however, as there were large numbers of potential food sources for each farm it was common for no feasible solutions to occur (indicated by zero observations being generated). As such, the minimum mass balance tolerance that resulted in a feasible solution was used. In the present study tolerances were between 0.2 and 2 % which are within the range used in previous studies with no bias introduced with larger tolerance levels (Benstead et al. 2006; Cole et al. 2011; Duffy et al. 2011; Stenroth et al. 2006)

Reporting the distribution of feasible solutions is recommended by Phillips et al. (2005) however, sources with large distributions tend to not reflect the number of observations (frequency) of the source contributions. To adequately represent the source contribution the distribution is supported by the mean ± SD. The distribution range is presented 1–99th percentiles as the full range is sensitive to the small observations present in the tails of the distribution (Phillips and Gregg 2003). Mean SI values of ponds ($n = 2$) were pooled together with the average representing the farm overall. The isotopic composition of potential food sources was examined in relation to whole animal and tail muscle. Additionally, the contribution of supplementary feed was examined in relation to zooplankton and phytoplankton.

A one-way ANOVA followed by Tukey's test on significant terms was conducted on the mean stable isotope values of whole animal and tail-muscle among farms. Significance level was accepted at $P < 0.05$.

## Results

### Stable isotope data analysis

There was an overall significant difference between farms for whole-body $\delta^{13}C$ ($F = 27.23$, d.f. $= 7$, $P < 0.05$), and $\delta^{15}N$ ($F = 42.65$, d.f. $= 7$, $P < 0.05$), and for tail-muscle $\delta^{13}C$ ($F = 117.30$, d.f. $= 7$, $P < 0.01$) and $\delta^{15}N$ ($F = 54.025$, d.f. $= 7$, $P < 0.01$) (Fig. 1).

Tukey post hoc comparisons of the farms indicate that there were no significant differences in whole-body $\delta^{13}C$ and $\delta^{15}N$ between Wondecla Farm and Topaz Farm or Mareeba Farm and Biboorha Farm. There were significant differences for $\delta^{13}C$ between Mareeba Farm and Wondecla Farm ($P < 0.01$) and Mareeba Farm and Topaz Farm ($P < 0.01$) and between Biboorha Farm and Wondecla Farm ($P < 0.01$). Whole-body $\delta^{15}N$ differed significantly between Mareeba Farm and Wondecla Farm ($P < 0.05$), Mareeba Farm and Topaz Farm ($P < 0.01$), Biboorah Farm and Wondecla Farm ($P < 0.01$) and for Biboorah Farm and Topaz Farm ($P < 0.01$). Tail-muscle isotopic values were significantly different ($P < 0.01$) except for $\delta^{13}C$ between Wondecla Farm and Topaz Farm ($P < 0.05$) and $\delta^{15}N$ between Mareeba Farm and Biboorha Farm ($P < 0.05$).

### Multi-source mixing model

Proportions of the different food sources that contribute to the isotopic signature of the crayfish were estimated using the multi-source mixing model. As no organic carbon values are present in diammonium phosphate (Biboorha Farm) or dolomite and lime (Topaz Farm) these were excluded from the model. Results show the majority of supplemental feed provided is assimilated by redclaw. Similarly plant material and periphyton predominantly contribute when present in relation to supplemental feed and/or natural pond biota. Source

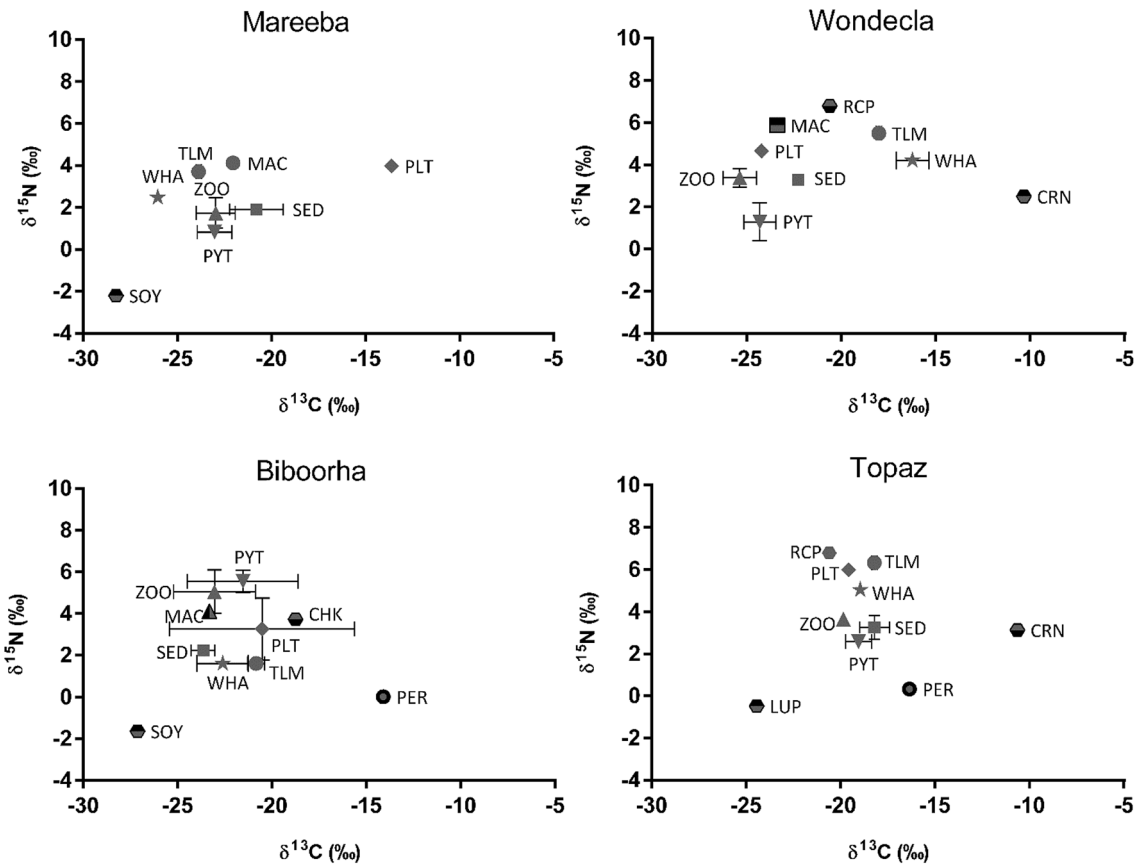


**Fig. 1** Isotopic signature of *C. quadricarinatus* and potential food sources sampled at four commercial redclaw farms. Plotted values are means, *error bars* represent ±SD. *WHA* whole animal, *PYT* phytoplankton, *ZOO* zooplankton, *SED* sediment, *PLT* plant material, *PER* periphyton, *MAC* macroinvertebrates, *LUP* lupin, *RCP* commercial redclaw pellet, *SOY* soybean, *CHK* chicken feed

contributions are presented in Table 1. Refer to Online Resources 1 (Fig. S1–4) for histograms generated of the feasible source contributions for each farm.

Whole-body: naturally occurring pond organisms

The highest contribution of naturally occurring pond biota occurred at the Mareeba Farm with zooplankton contributing between 0–57 % (mean ± SD; 17.4 ± 14.3 %) and 0–44 % (11.9 ± 10.7 %) for phytoplankton. Additionally, the contribution of macro-invertebrates was the highest for all farms ranging between 1 and 49 % (22.6 ± 10.7 %). Contribution of naturally occurring pond biota was lower at the remaining farms particularly at Wondecla Farm where zooplankton had a contribution of only 0–11 % (2.90 ± 2.80 %) and phytoplankton 0–9 % (2.10 ± 2.20 %).

Whole-body: supplementary feed

Supplemental feed was readily consumed at most of the farms. Corn used at Mareeba Farm had a contribution of between 25 and 52 % (mean ± SD; 37.7 ± 6.20 %). The contribution of supplemental feed was quite high in relation to other sources at Wondecla Farm with the commercial redclaw pellet of 11–41 % (29.9 ± 6.60 %) and corn ranging between 45 and 53 % (49.0 ± 1.70 %). While the redclaw pellet had a contribution at the Topaz Farm of 0 to 82 % (26.1 ± 19.1 %) plant material was greater with a feasible contribution of between 0 and 86 % (43.9 ± 19.5 %) and lupin contributing very little 0–12 % (1.60 ± 1.70 %). Conversely, chicken feed at the Biboorha Farm did not contribute greatly to the diet with a

**Table 1** Summary of the average source contributions (%; ±SD) to the diet of farmed *C. quadricarinatus*

| Source (Relative % contribution) | Farm | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mareeba | | Biboorha | | Wondelca | | Topaz | |
| | Whole animal | Tail muscle | Whole animal | Tail muscle | Whole animal | Tail muscle | Whole animal | Tail muscle |
| Naturally occurring pond biota | | | | | | | | |
| Zooplankton | 17.4 ± 14.3 | 6.90 ± 6.20 | 9.00 ± 7.50 | 13.0 ± 11.0 | 2.90 ± 2.80 | 1.20 ± 1.40 | 6.10 ± 5.60 | 2.90 ± 2.90 |
| Phytoplankton | 11.9 ± 10.4 | 4.20 ± 4.00 | 8.30 ± 7.50 | 14.8 ± 12.2 | 2.10 ± 2.20 | 0.80 ± 1.10 | 4.50 ± 4.20 | 2.00 ± 2.20 |
| Macro-invertebrates | 22.6 ± 10.7 | 70.40 ± 4.40 | 10.2 ± 8.90 | 12.3 ± 10.47 | 8.40 ± 7.40 | 3.80 ± 3.60 | – | – |
| Supplemental feed | | | | | | | | |
| Corn | – | – | – | – | 49.0 ± 1.70 | 27.6 ± 1.10 | 8.20 ± 3.10 | 8.30 ± 3.10 |
| Redclaw Pellet | – | – | – | – | 29.9 ± 6.60 | 63.0 ± 3.70 | 26.1 ± 19.1 | 18.2 ± 16.1 |
| Soybean | 37.7 ± 6.20 | 15.0 ± 2.40 | 25.8 ± 8.80 | 6.00 ± 4.90 | – | – | – | – |
| Chicken feed | – | – | 10.0 ± 8.80 | 16.40 ± 12.90 | – | – | – | – |
| Lupin | – | – | – | – | – | – | 1.60 ± 1.70 | 0.60 ± 0.90 |
| Additional sources | | | | | | | | |
| Sediment | 7.70 ± 6.90 | 2.60 ± 2.60 | 15.6 ± 13.7 | 10.70 ± 9.40 | 3.90 ± 3.70 | 1.70 ± 1.80 | 6.50 ± 5.90 | 3.00 ± 3.10 |
| Periphyton | – | – | 9.40 ± 6.00 | 11.50 ± 7.20 | – | – | 3.00 ± 2.90 | 1.30 ± 1.50 |
| Plant material | – | – | 11.6 ± 10.3 | 15.20 ± 13.20 | 4.60 ± 4.20 | 2.00 ± 2.10 | 43.9 ± 19.5 | 63.6 ± 16.4 |

Blank cells indicate source material that was not used at a particular site

range of 0–37 % (10.0 ± 8.80 %) compared with soybean which ranged between 5 and 46 % (25.8 ± 8.80 %). The contribution of the other food sources sampled (naturally occurring pond biota, periphyton, plant material, etc.) were less easily distinguished at this farm.

## Assimilation of sources in tail-muscle

When the mixing model is applied to the tail-muscle the potential food source that is being assimilated by the crayfish for growth become more identifiable as there is a distinct difference in the contribution value to that of the whole-body (Table 1). At the Mareeba Farm where zoo- and phytoplankton had high contributions for whole-body this fell to 0–26 % (mean ± SD; 6.90 ± 6.20 %) and 0–16 % (4.20 ± 4.00 %), respectively. Macro-invertebrates increased to a contribution of between 60 and 81 % (70.4 ± 4.40 %). Soybean was also readily assimilated in the tail muscle at a range of 10–20 % (15.0 ± 2.40 %). At Wondecla Farm the contribution of the redclaw pellet was 55–72 % (63.0 ± 3.70 %), and corn the second highest source contribution at 25–30 % (27.6 ± 1.10 %). Naturally occurring pond biota and macro-invertebrates contributed less than 25 %. Plant material at the Topaz Farm remained high contributing between 13 and 86 % (63.6 ± 16.4 %) with the redclaw pellet falling slightly to 0–69 % (18.2 ± 16.1 %). Sources utilised for growth at Biboorha Farm are less distinguishable, however, soybean contribution was 0–20 % (6.0 ± 4.90 %) and chicken feed 0–53 % (16.4 ± 12.9 %). Overall, the contribution of zooplankton and phytoplankton in relation to tail-muscle was less than that observed in the whole-body analysis.

## Contribution of supplementary feed to primary productivity

Stable isotope values for supplementary feed were also analysed in relation to their contribution to zooplankton and phytoplankton productivity (Table 2). As the Mareeba Farm only had one supplemental feed source (soybean) the minimum of three isotope mixtures required by the mixing model were not met and the farm was therefore excluded from this analysis (Phillips and Gregg 2003). For the remaining farms the results clearly show that supplemental feed acts as an additional nutrient source for primary pond productivity. The contribution of commercial chicken feed at Biboorha Farm ranged between 64–83 % (mean ± SD; 72.6 ± 4.70 %) for zooplankton and 71–99 % (83.4 ± 6.90 %) for phytoplankton, similarly, results for the redclaw pellet at Wondecla Farm were also high, 72–98 % (83.1 ± 6.50 %) and 58–97 % (50.0 ± 9.50 %), respectively. At the Topaz Farm, the redclaw pellet ranged from 34 to 74 % (54.8 ± 11.2 %) and 10–52 % (31.0 ± 11.2 %) with lupin comparable with ranges of 12–42 % (27.3 ± 8.10 %) for zooplankton and 23–54 % (38.7 ± 8.10 %) for phytoplankton.

**Table 2** Feasible source contributions (%) of supplementary feed to primary productivity*

| Source | Farm | | | | | |
|---|---|---|---|---|---|---|
| | Biboorha | | Wondecla | | Topaz | |
| | Phytoplankton (%) | Zooplankton (%) | Phytoplankton (%) | Zooplankton (%) | Phytoplankton (%) | Zooplankton (%) |
| Corn | – | | 0–29 | 0–20 | 18–42 | 6–30 |
| Redclaw pellet | – | – | 58–97 | 72–98 | 10–52 | 34–76 |
| Soybean | 0–20 | 16–30 | – | – | – | – |
| Chicken pellet | 71–99 | 64–83 | – | – | – | – |
| Lupin | – | – | – | – | 23–54 | 12–42 |
| Hay | 0–17 | 0–11 | 0–40 | 0–27 | – | – |

Blank cells indicate source material that was not used at a particular site

* Minimum of three sources required to calculate feasible contributions with Mareeba Farm not meeting this requirement

## Discussion

This study identified for the first time the feed sources contributing to the diet of redclaw grown in semi-intensive commercial pond aquaculture. SIA demonstrated that while supplemental feed is a primary nutrient source contributing to the diet of redclaw; the availability of naturally occurring sources appears to be a factor in the relative contribution of the supplemental feed to the diet. For, e.g. natural vegetation contributed a significant proportion of redclaw diet on some farms even though there was a number of supplementary sources provided (lupin, corn and commercial redclaw pellet). Further, raw lupin, a commonly used feed, had the lowest contribution for all supplemental feed sources sampled. More importantly, a clear contribution of the redclaw pellet, chicken feed, lupin, and soybean to both zoo- and phytoplankton production was expressed when the multi-source mixing model was applied to supplementary feed in relation to primary productivity. Chicken feed and lupin which were shown to provide little direct contribution to the diet of the redclaw, rather acting as direct C and N sources for primary pond productivity. These results have direct implication on the effectiveness of current feeding practices in Australian redclaw pond aquaculture, highlighting the benefit of providing a water stable formulated feed suited to the requirements of redclaw.

Current intensive and semi-intensive crayfish farming methods rely on the availability of natural food items as an important source of nutrition for freshwater crayfish species (Jones 1989; Meakin et al. 2009; Momot 1995). Using SIA Duffy et al. (2011) found that *Cherax destructor* consumed natural food items even in the presence of formulated feed. Similarly, zooplankton and biofilm (comprised of organisms such as flagellates, ciliates, rotifers, and nematodes) improved survival and growth of juvenile redclaw (Viau et al. 2012) and juvenile *C. destructor* (Verhoef et al. 1998). Production in ponds is initially stimulated with chemical fertilizers and/or organic matter (e.g. hay) (Jones et al. 1996) and some farms will routinely add additional fertiliser, e.g. dolomite and lime, during the culture period. Stimulation of primary pond productivity is important in semi-intensive culture, particularly when supplementary feeding doesn't provide adequate nutrition, and also because of their role in nutrient cycling and the influence on water quality (Moriarty 1997). Results from the current study corroborate this understanding as zoo- and phytoplankton, associated periphyton and plant material were also found to contribute to a similar or greater degree as supplemental feed. Conversely, the role of primary productivity in a system where adequate supplemental feed is not available is also demonstrated, particularly at the Mareeba Farm. Here, SIA and the multi-source mixing model show that the provision of soybean was secondary to the contribution of naturally occurring pond biota, in particular macro-invertebrates. The Mareeba Farm had little vegetation in and around the ponds (restricted by a steep pond edge) and the depth was quite shallow at $\sim$0.6 m in comparison to the depth of the other farms ($\sim$1.2 m). As crayfish are benthic in nature it is possible that the shallower ponds resulted in increased consumption of naturally occurring pond organisms due to their accessibility within the water column (Duffy et al. 2011; Meakin et al. 2009). Migration of *Daphina* spp. in shallow lakes has shown to result in very high densities of zooplankton in vegetated littoral zones (Burks et al. 2002; Burns 2000) and could allow for increased consumption by crayfish (Meakin et al. 2009). Indeed, redclaw are known to switch to a more herbivorous diet as they grow larger (Giling et al. 2009; Momot 1995) and *C. destructor* of up to 45 g have been shown to actively respond to, and, prefer live zooplankton (Meakin et al. 2009). Crayfish from the Mareeba Farm were the largest by weight (54.29 $\pm$ 14.36 g ($n = 22$) and unlike other farms where multiple feed sources was supplied soybean was the only alternative food source provided. The contribution of macro-invertebrates to the diet is thought to be a reflection of the pond depth, lack of vegetation and alternative food sources.

Plant-based sources are palatable and readily consumed by redclaw (Cronin et al. 2002) therefore the use of raw plant-based feeds would be expected to be reflected in the isotopic signatures of redclaw. However, this was not the case for a number of the supplementary sources (i.e. chicken feed, lupin, corn). While the commercial redclaw pellet is readily consumed and digested (Conrado, Pichette & Pirozzi; unpub. data 2014) it has an inherent low water stability (Gamble & Pirozzi, unpub data, 2014). Instead of the intact feed being directly consumed the disintegration of pellets and decomposing raw feed may form a nutrient-source and/or substrate for biofilm to accumulate (Loya-Javellana et al. 1993; Salame and Rouse 2000; Viau et al. 2012; Wood et al. 2012). Sources such as sediment, plant material and tyre scrapings (periphyton) were also found to contribute to a small degree to the diet of redclaw. Additionally benthic detritus and associated micro-organisms (biofilm) have been suggested to be a source of nutrition for juvenile redclaw (Jones 1989),

*Procambarus clarkii* (Cronin et al. 2002), *C. destructor* (Giling et al. 2009) and *Pacifastacus leniusculus* (Bondar et al. 2005). It is unknown whether biofilm is improving the nutritional profile of the decomposing feed, or is an important dietary source for crayfish and is a concept that should be investigated further. Similarly, it is unknown if sources such as pond sediment and plant material have any nutritional benefit to the redclaw sampled at this farm, or whether accumulation of biofilm is improving the nutritional profile and palatability of such sources and therefore increasing intake.

In natural systems C. *destructor* and *C. cainii* are known to consume predominantly animal matter, such as small fishes and gastropods in summer; however, during winter, diets shift towards a more herbivorous predominance with crayfish consuming greater amounts of *Melaleuca spp*. when the abundance of high-protein food sources became limited (Beatty 2006). There are no studies that have documented this dynamic in extensive or semi-intensive aquaculture pond systems. Species richness and abundance of naturally occurring pond biota will likely vary seasonally, particularly at farms where water is sourced from natural river systems such as Biboorha Farm, which obtains water directly from the Barron River, and at farms with little to no filtration of incoming water. However, Stenroth et al. (2006) reported that there were no significant changes in isotopic signatures in the months or years of their study which investigated the use of stable isotopes as an indicator of the diet for the signal crayfish (*Pacifastacus leniusculus*) in the lakes of southern Sweden. The authors suggested that the crayfish either had a consistent diet throughout the seasons or that crayfish muscle tissue had a turnover rate slow enough to mask any differences of the diet during those months in-between sampling. Given the variability of pond productivity and performance of supplementary feed a nutritionally complete feed that is water stable, digestible by redclaw and therefore utilised more efficiently will result in improved food conversion ratios (FCRs), reduce nutrient loading in pond aquaculture systems and decrease harvest times, all of which have direct cost benefits to the farmer. Implementing more efficient feeds and feed practices for redclaw aquaculture will help to promote the growth of the industry.

When studying diet and trophic interactions the $\delta^{15}N$ enrichment of sources needs to be considered. In crustaceans $\delta^{15}N$ is reported to be approximately 2 ‰ (Vanderklift and Ponsard 2003) with values from Mareeba Farm and Biboorha Farm fitting this standard. Enrichment values for Wondecla Farm and Topaz Farm are higher at 4.21 and 5.04 ‰, respectively, however, $\delta^{15}N$ values of 5.7 ‰ (Duffy et al. 2011) and 6 ‰ (Rudnick and Resh 2005) have previously been reported for crayfish. The $\delta^{15}N$ of the redclaw may be a reflection of the contribution of the redclaw pellet and plant material to the diet as they had $\delta^{15}N$ values of 6.78 ‰ and, respectively, 5.99 ‰. As the isotopic signatures identified for redclaw in the current study were determined from whole-body and tail-muscle samples, which are generally tissues that integrate isotopic signatures from the diet over a longer time period (Bodin et al. 2007), short term changes in feedstuffs should therefore have little influence on stable isotope signatures. The contribution of feed sources generally decreased slightly for tail-muscle compared to whole-body. There were some exceptions, however, particularly the contribution of the redclaw pellet at Wondecla Farm (63.0 ± 3.70 vs 29.9 ± 6.60 %), plant matter at Topaz Farm (63.6 ± 16.4 vs 43.9 ± 19.5 %) and macro-invertebrates at Mareeba Farm (70.40 ± 4.40 vs 22.6 ± 10.7 %). The reason for these increases are unknown and it is speculated that the utilisation efficiency and assimilation of these sources is different in muscle tissue compared to Whole animal (e.g. Stenroth et al. 2006). Regardless, those sources with the greatest contribution at each farm were the same whether looking at whole-body or tail muscle (Table 1).

Redclaw are known to have cannibalistic tendencies, particularly when individuals are most vulnerable when moulting (Barki et al. 1997). Jones and Ruscoe (2000) commented that high stocking densities (maximum density recommended to be between 9 and 15 $m^2$), insufficient supplemental feeding, and limited shelter options are other factors that can influence such behaviour. The farms in this study initially stock according to the industry recommendation of five redclaw per $m^2$ in a standard 1000 $m^2$ pond with sufficient shelter provided (Stevenson and Keast 2014). This is a relatively low stocking density but it is possible that some cannibalism still occurs. Regardless, stable isotope analysis cannot differentiate nutrient contribution through cannibalistic events and in such circumstances radio labelling techniques in a controlled trial would be more appropriate.

## Conclusion

Both naturally occurring pond biota and supplemental feed contributed to the tissue composition of redclaw indicating the omnivorous nature of this species. In general, supplemental feed had a greater contribution to the diet than zooplankton, phytoplankton or macro-invertebrates. However, the contribution varied with the type of feed provided and the availability of alternative sources. Given the reliance and predominant use of relatively cheap and readily available raw feeds there is surprisingly little information available in relation to their suitability for redclaw. SIA techniques demonstrated that raw feeds such as soybean and corn were consumed to some degree but others like lupin were not. Further research into the use of different processing of raw feed could be beneficial (e.g. dehulling) give current feed practices; however, ultimately a move towards a nutritionally appropriate formulated feed is required to significantly improve redclaw production efficiencies. Some supplemental feeds sampled in this study such as commercial chicken and lesser extent redclaw pellets were shown to indirectly provide a nutrient supply for redclaw, instead providing a direct nutrient source for primary pond productivity. While maintaining healthy and productive natural pond biota is important to optimise growth of redclaw, in this case, the use of cheaper fertilisers would likely be more cost effective. The development of a formulated feed of an appropriate nutrient specification that meets the nutritional requirements of redclaw and has good water stability is vital for the industry to grow, particularly with the increase towards more intensive culture systems. Such a move would reduce the reliance on primary productivity and supplemental feed ultimately improving current feed management strategies and ensuring production security.

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